REMARKS

Applicants respectfully request that new claims 37-58 be added to claims 20-36, which are currently pending in this application.

New claims 37-58 were derived from claims 20-36 are fully supported by the specification of the application, for example as follows:

Limitations in Applicants' claim	**Support in Application (regular text refers to U.S. and PCT applications, *italicized text refers to U.K. application*)**
37 and 48. A method for producing	This invention relates to the generation of animals including but not being limited to genetically selected and/or modified animals, and to cells useful in their generation. (Pg. 1, lines 4-7) *(Pg. 1, lines 3-6)*. . . . all animals produced from embryos prepared by the invention should transmit the relevant genetic modification through the germ line as each animal is derived from a single nucleus; (Pg. 6, lines 9-12) *(Pg. 6, lines 6-9)*. 13. A method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as claimed in any preceding claim; (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. (Original claim 13).

Limitations in Applicants' claim	**Support in Application (regular text refers to U.S. and PCT applications, *italicized text refers to U.K. application*)**
a mammalian cultured inner cell mass cell	Alternatively, or additionally, it may be possible for increased yields of viable embryos to be achieved by means of the present invention by clonal expansion of donors and/or if use is made of the process of serial (nuclear) transfer. A limitation in the presently achieved rate of blastocyst formation may be due to the fact that a majority of the embryos do not "reprogram" (although an acceptable number do). If this is the case, then the rate may be enhanced as follows. Each embryo that does develop itself can be used as a nuclear donor at the 32-64 cell state; alternatively, inner cell mass cells can be used at the blastocyst stage. (Page 16, lines 9-20) *(Pg. 15, line 32 through pg. 16, line 9).*
by nuclear transfer comprising	In the method of the invention described above, a diploid nucleus is transferred from a donor into the enucleated recipient oocyte. (Page 7, lines 16-18) *(Pg. 7, lines 13-15).* After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating condition. (Page 10, lines 18-21) *(Pg. 10, lines 16-29).* Most conveniently, nuclear transfer is effected by fusion. (Page 10, lines 28-29) *(Pg. 10, lines 26-27).*
(i) inserting a nucleus of a diploid non-human mammalian differentiated somatic cell in the G1 phase of the cell	The mitotic cell cycle has four distinct phases, G, S, G2 and M. The beginning event in the cell cycle, called start, takes

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cycle into an	place in the G1 phase and has a unique function. The decision or commitment to undergo another cell cycle is made at start. Once a cell has passed through start, it passes through the remainder of the G1 phase, which is the pre-DNA synthesis phase. (Page 7, lines 26-32) *(Page 7, lines 22-28).* . . . therefore donors may be either in the G1 phase or preferably, as is the subject of our co-pending PCT patent application No. PCT/GB96/02099 filed today (claiming priority from GB 9517780.4), in the G0 phase of the cell cycle. (Page 7, line 20-24) *(Page 7, lines 17-20, except that "our co-pending PCT application No. PCT/GB96/02099" is replaced with "our co-pending UK patent application").* Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. (Page 8, lines 13-19) *(Page 8, lines 9-15).* In order to maintain the correct ploidy of the reconstructed embryo the donor nucleus must be diploid (i.e., in the G0 or G1 phase of the cell cycle) at the time of fusion. (Page 10, lines 21-24) *(Page 10,*

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	lines 19-22).
unactivated,	After enucleation, the donor nucleus is introduced either by fusion to donor cells under conditions which do not induce oocyte activation or by injection under non-activating conditions. (Page 10, lines 18-21) *(Page 10, lines 16-19).* In a preferred embodiment of the invention, fusion of the oocyte karyoplast couplet is accomplished in the absence of activation by electropulsing in 0.3M mannitol solution or 0.27M sucrose solution; alternatively the nucleus may be introduced by injection in a calcium free medium. The age of the oocytes at the time of fusion/injection and the absence of calcium ions from the fusion/injection medium prevent activation of the recipient oocyte. (Page 12, lines 1-8) *(Page 11, line 32, through page 12, line 5).*
enucleated	It is preferred that the recipient be enucleated. (Page 9, line 7) *(Page 9, line 4).*
metaphase II-arrested	Recipient cells useful in the invention are enucleated oocytes which are arrested in the metaphase of the second meiotic division. (Page 8, lines 29-31) *(Page 8, lines 26-28).*
non-human mammalian oocyte	According to a first aspect of the present invention there is provided a method of reconstituting an animal embryo, the method comprising transferring a diploid nucleus into an oocyte which is arrested in the metaphase of the second meiotic division without concomitantly activating the oocyte, keeping the nucleus exposed

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	to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth and subsequently activating the reconstituted embryo while maintaining correct ploidy. At this stage, the reconstituted embryo is a single cell. In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 1-28) *(Page 4, line 33, through page 5, line 25).*
of the same species to reconstruct an embryo;	The invention is equally applicable in the production of transgenic, as well as non-transgenic animals. (Page 5, lines 30-31) *(Page 5, lines 27-28).*
(ii) activating the resultant-reconstructed embryo;	When it is time for activation, any conventional or other suitable activation protocol can be used. (Page 13, lines 5-6) *(Page 13, lines 1-2).*

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	The optimum period of time before activation varies from species to species and can readily be determined by experimentation. (Page 12, lines 30-32) *(Page 12, lines 27-29)*.
(iii) culturing said activated, reconstructed embryo;	(4) culture, *in vivo* or *in vitro*, to blastocyst; (Page 19, line 24) *(Page 19, line 13)*.
(Claim 48 only) to form a cultured embryo that can develop to term when transferred to a host mammal of the same species; and	keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth . . . . (Page 5, lines 6-9) *(Page 5, lines 4-7)*. Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. A preferred source of cells is disclosed in our co-pending PCT patent application No. PCT/GB95/02095, published as WO 96/07732. It is believed that all such normal cells contain all of the genetic information required for the production of an adult animal. The present invention allows this information to be provided to the developing embryo by altering chromatin structure such that the genetic material can re-direct development. (Page 8, lines 13-27) *(Page 8, lines 9-24, except "our co-pending PCT application No. PCT/GB95/02095, published as WO*

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	96/07732" is replaced by "our co-pending UK patent application No. 9417831.6"). Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12 lines 22-28) *(Page 12, lines 19-25).* According to a second aspect of the invention, there is provided a viable reconstituted animal embryo prepared by a method as described previously. According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed. Step (a) has been described in depth above.

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	The second step, step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. This may be done directly or indirectly. In direct development, the reconstituted embryo from step (a) is simply allowed to develop without further intervention beyond any that may be necessary to allow the development to take place. In indirect development, however, the embryo may be further manipulated before full development takes place. For example, the embryo may be split and the cells clonally expanded, for the purpose of improving yield. (Page 15, line 15, through page 16, line 7) *(Page 15, lines 4-30).* According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 23-24) *(Page 20, lines 12-13).* Reconstructed embryos were cultured in the ligated oviduct of a temporary recipient ewe for 6 days, recovered and assessed for development. . . . Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn or synchronised final recipient blackface ewes. (Page 27, line 12, through page 28, line 4) *(Page 27, lines 2-27).*
(iv) isolating and culturing inner cell mass cells obtained from said cultured activated, reconstructed embryo to obtain	Alternatively, or additionally, it may be possible for increased yields of viable embryos to be achieved by means of the

Limitations in Applicants' claim	**Support in Application (regular text refers to U.S. and PCT applications, *italicized text refers to U.K. application*)**
a cultured inner cell mass cell	present invention by clonal expansion of donors and/or if use is made of the process of serial (nuclear) transfer. A limitation in the presently achieved rate of blastocyst formation maybe due to the fact that a majority of the embryos do not "reprogram" (although an acceptable number do). If this is the case. then the rate may be enhanced as follows. Each embryo that does develop itself can be used as a nuclear donor at the 32-64 cell state; alternatively, inner cell mass cells can be used at the blastocyst stage. (Page 16, lines 9-20) *(Page 15, line 32, through page 16, line 9).*
wherein the cultured inner cell mass cell can differentiate.	keeping the nucleus exposed to the cytoplasm of the recipient for a period of time sufficient for the reconstituted embryo to become capable of giving rise to a live birth . . . . (Page 5, lines 6-9) *(Page 5, lines 4-7).* Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. The only limitation is that the donor cells have normal DNA content and be karyotypically normal. A preferred source of cells is disclosed in our co-pending PCT patent application No. PCT/GB95/02095, published as WO 96/07732. It is believed that all such normal cells contain all of the genetic information required for the production of an adult animal. The present invention allows this information to be provided to the developing embryo by altering

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	chromatin structure such that the genetic material can re-direct development. (Page 8, lines 13-27) *(Page 8, lines 9-24, except "our co-pending PCT application No. PCT/GB95/02095, published as WO 96/07732" is replaced by "our co-pending UK patent application No. 9417831.6").*
	Subsequently, the fused reconstructed embryo, which is generally returned to the maturation medium, is maintained without being activated so that the donor nucleus is exposed to the recipient cytoplasm for a period of time sufficient to allow the reconstructed embryo to become capable, eventually, of giving rise to a live birth (preferably of a fertile offspring). (Page 12 lines 22-28) *(Page 12, lines 19-25).* According to a second aspect of the invention, there is provided a viable reconstituted animal embryo prepared by a method as described previously. According to a third aspect of the invention, there is provided a method of preparing an animal, the method comprising: (a) reconstituting an animal embryo as described above; and (b) causing an animal to develop to term from the embryo; and (c) optionally, breeding from the animal so formed.

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	Step (a) has been described in depth above.
	The second step, step (b) in the method of this aspect of the invention is to cause an animal to develop to term from the embryo. This may be done directly or indirectly. In direct development, the reconstituted embryo from step (a) is simply allowed to develop without further intervention beyond any that may be necessary to allow the development to take place. In indirect development, however, the embryo may be further manipulated before full development takes place. For example, the embryo may be split and the cells clonally expanded, for the purpose of improving yield. (Page 15, line 15, through page 16, line 7) *(Page 15, lines 4-30)*. According to a fourth aspect of the invention, there is provided an animal prepared as described above. (Page 20, lines 23-24) *(Page 20, lines 12-18)*.
	Reconstructed embryos were cultured in the ligated oviduct of a temporary recipient ewe for 6 days, recovered and assessed for development. [Table 4] Table 5 shows induction of pregnancy following transfer of all morula/blastocyst stage reconstructed embryos to the uterine horn or synchronised final recipient blackface ewes. (Page 27, line 12, through page 28, line 4) *(Page 27, lines 2-27)*.

Limitations in Applicants' claim	**Support in Application (regular text refers to U.S. and PCT applications,** ***italicized text refers to U.K. application*****)**
38 and 49. The method of claim 37 (or 48), wherein said diploid non-human mammalian differentiated cell in the G1 phase of the cell cycle is a fibroblast cell.	Reconstruction of bovine embryos using "MAGIC" procedure In preliminary experiments this technique has been applied to the reconstruction of bovine embryos using primary fibroblasts synchronised in the G0 phase of the cell cycle by serum starvation for five days. The results are summarized in Table 3. Table 3 shows development of bovine embryos reconstructed by nuclear transfer of serum starved (G0) bovine primary fibroblasts into enucleated unactivated MII oocytes. Embryos were reconstructed at 24 hpm and the fused couplets activated at 42 hpm. Fused couplets were incubated in nocodazole (5μg/ml)in M2 medium for 1 hour prior to activation and 5 hours post activation. (Page 25, line 31, through page 26, line 10) *(Page 25, lines 15-27, except that the heading is not present).*
39 and 50. The method of claim 37 (or 48), comprising culturing said activated, reconstructed embryo to form a blastocyst, and culturing inner cell mass cells obtained from said blastocyst to produce a cultured inner cell mass cell.	In indirect development, however, the embryo may be further manipulated before full development takes place. For example, the embryo may be split and the cells clonally expanded, for the purpose of improving yield. Alternatively, or additionally, it may be possible for increased yields of viable embryos to be achieved by means of the present invention by clonal expansion of donors and/or if use is made of the process of serial (nuclear) transfer. A limitation in the presently achieved rate of blastocyst formation maybe due to the fact that a majority of the embryos do not

Limitations in Applicants' claim	**Support in Application (regular text refers to U.S. and PCT applications, *italicized text refers to U.K. application*)**
	"reprogram" (although an acceptable number do). If this is the case. then the rate may be enhanced as follows. Each embryo that does develop itself can be used as a nuclear donor at the 32-64 cell state; alternatively, inner cell mass cells can be used at the blastocyst stage. (Page 16, lines 3-20) *(Page 15, line 26 through page 16, lines 9).*
40 and 51. The method of claim 37 (or 48), wherein said nucleus is genetically modified. 41 and 52. The method of claim 40 (or 51), wherein the genome of said genetically modified nucleus comprises an insertion, deletion, or modification. 42 and 53. The method of claim 41 (or 52), wherein said genetically modified nucleus comprises an exogenous DNA. 44 and 55. The method of claim 43 (or 54), wherein said nucleus comprises at least one genetic modification.	It should be noted that the term "transgenic", in relation to animals, should not be taken to be limited to referring to animals containing in their germ line one or more genes from another species, although many transgenic animals will contain such a gene or genes. Rather, the term refers more broadly to any animals whose germ line has been the subject of technical intervention by recombinant DNA technology. So, for example, an animal in whose germ line an endogenous gene has been deleted, duplicated, activated or modified is a transgenic animal for the purposes of this invention as much as an animal to whose germ line an exogenous DNA sequence has been added. In embodiments of the invention in which the animal is transgenic, the donor nucleus is genetically modified, The donor nucleus may contain one or more transgenes and the genetic modification may take place prior to nuclear transfer and embryo reconstitute. Although microinjection, analogous to injection into the male or female pronucleus of a zygote, may be used as a method of genetic modification, the invention is not limited to that methodology: mass transformation or transfection techniques can also be used

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	e.g. electroporation, viral transfection or lipofection. (Page 6, line 24, through page 7, line 14) *(Page 6, line 21, through page 7, line 11).* A method as claimed in any one of claims 1 to 3, in which the donor nucleus is genetically modified. (Original claim 4) *(See claim 4).*
43 and 54. The method of claim 37 (or 48), wherein said nucleus is isolated from a mammal selected from the group consisting of sheep, cows, pigs, horses, rabbits, rodents, mice, and rats. 47 and 58. Method of claim 37 (or 48), wherein the cultured inner cell mass cell is a cow or pig inner cell mass cell.	In principle, the invention is applicable to all animals, including birds such as domestic fowl, amphibian species and fish species. In practice, however, it will be to non-human animals, especially non-human mammals, particularly placental mammals, that the greatest commercially useful applicability is presently envisaged. It is with ungulates, particularly economically important ungulates such as cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 14-28) *(Page 4, line 33, through page 5, line 25).* A method as claimed in claim 2, in which the animal is a cow or bull, pig, goat, sheep, camel or water buffalo. (Original claim 3) *(See claim 3).*
45 and 56. The method of claim 37 (or 48), wherein said nucleus is isolated	It is with ungulates, particularly economically important ungulates such as

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from an ungulate.	cattle, sheep, goats, water buffalo, camels, and pigs that the invention is likely to be most useful, both as a means for cloning animals and as a means for generating transgenic animals. It should also be noted that the invention is also likely to be applicable to other economically important animal species such as, for example, horses, llamas or rodents, e.g. rats or mice, or rabbits. (Page 5, lines 20-28) *(Page 5, lines 17-25)*. A method as claimed in claim 1, in which the animal is an ungulate species. (Original claim 2) *(See claim 2)*.
46 and 57. The method of claim 37 (or 48), wherein said diploid non-human mammalian differentiated cell in the G1 phase of the cell cycle is expanded in vitro prior to step (i).	Subject to the above, it is believed that there is no significant limitation on the cells that can be used in nuclear donors: fully or partially differentiated cells or undifferentiated cells can be used as can cells which are cultured *in vitro* or abstracted *ex vivo*. (Page 8, lines 13-17) *(Page 8, lines 9-13)*.

No new matter is added by the new claims. Applicants respectfully request that new claims 37-58 be considered with the previously pending claims 20-36.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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